

El cont.

(a) has no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding amino acid positions in a CTL epitope which is

LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),

QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO:3),

KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or

LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35), or

(b) has no more than one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),

DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),

LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),

SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or

ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42),

wherein said molecule comprises at least eight amino acids and less than 50 amino acids, with the provisos that (i) when said selected CTL epitope is SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), then said molecule comprises from at least eight amino acids to less than 25 amino acids, (ii) when said selected CTL epitope is LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2) then said molecule comprises at most ten amino acids, and (iii) when said selected CTL epitope is DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54), then said molecule comprises at most nine amino acids.

23. **(Unchanged)** The molecule of claim 22, wherein the isolated peptide has less than 20 amino acids.

24. **(Unchanged)** The molecule of claim 22, wherein the isolated peptide has from 8 to 12 amino acids.

25. **(Unchanged)** The molecule of claim 22, wherein the isolated peptide has 9 or 10 amino acids.

E²
30. **(Unchanged)** The molecule of claim 22, 23, 24, or 25, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2).

32. **(Unchanged)** The molecule of claim 22, 23, 24, or 25, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO:3).

36. **(Unchanged)** The molecule of claim 22, 23, 24, or 25, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28).

40. **(Unchanged)** The molecule of claim 22, 23, 24, or 25, wherein the isolated molecule has a sequence that has no more than a total of one amino acid substitution, deletion or insertion at the corresponding position as in LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35).

44. **(Unchanged)** An immunogenic composition that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes (CTL) comprising molecule which comprises a peptide having a sequence that has no more than a total of a total of two amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),

LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),

QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO:3),

KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or

LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35) or

has no more than a total of one substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),

LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),
SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or
ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42).

45. **(Unchanged)** The immunogenic composition of claim 44, wherein the immunogenic composition further comprises a label selected from the group consisting of a radioactive label, an enzymatic label, and a fluorescent label.
46. **(Unchanged)** The immunogenic composition of claim 44, wherein the immunogenic composition further comprises a solid matrix.
47. **(Unchanged)** The immunogenic composition of claim 44, wherein the immunogenic composition further comprises a carrier molecule.
48. **(Unchanged)** The immunogenic composition of claim 44, wherein the carrier molecule comprises a protein or an immunogenic lipid.
49. **(Unchanged)** The immunogenic composition of claim 44, wherein the immunogenic composition further comprises a T-helper lymphocyte epitope.
50. **(Unchanged)** The immunogenic composition of claim 44, wherein the immunogenic composition further comprises an additional peptide.
51. **(Unchanged)** The immunogenic composition of claim 50, wherein the additional peptide has a sequence that has no more than a total of two amino acid substitutions, deletions or insertions at the corresponding positions as in KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28).
52. **(Unchanged)** A method of stimulating a cytotoxic T-lymphocyte (CTL) response to an hepatitis C viral immunogen, comprising contacting an HLA class I-restricted cytotoxic T

lymphocyte with a composition comprising a peptide that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes comprising a sequence that has no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),
LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),
QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO: 3),
KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or
LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35) or

has no more than a total of one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),
DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),
LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),
SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or
ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42).

53. (Unchanged) The method of claim 52, wherein the contacting occurs in a mammal.
54. (Unchanged) The method of claim 52, wherein the mammal is free of HCV disease, is a carrier of HCV, or is afflicted with HCV disease.
55. (Unchanged) The method of claim 52, wherein the contacting occurs *in vitro*.
56. (Unchanged) A method of detecting cytotoxic T cells that respond to a T cell epitope of hepatitis C virus (HCV), the method comprising the steps of:
- (a) preparing HLA class I-restricted cytotoxic T cells;
 - (b) preparing HLA class-I matched and -mismatched target cells;

(c) containing separately matched and mismatched target cells with a composition comprising a peptide that induces an HCV-specific response in cytotoxic T lymphocytes having the sequence that has no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),
LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),
QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO: 3),
KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or
LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35) or

has no more than a total of one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),
LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),
SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or
ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42);

(d) combining the cytotoxic T cells separately with the matched and mismatched target cells; and

(e) measuring cytolysis.

57. **(Unchanged)** The method of claim 56, wherein the cytotoxic T cells are combined with HLA class I-matched lymphocytes.

58. **(Unchanged)** A pharmaceutical composition comprising a peptide that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes having a sequence that has no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),
LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),
QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO: 3),

KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or
LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35) or
has no more than a total of one single amino acid substitution, deletion or insertion at the
corresponding amino acid positions as in a CTL epitope which is

DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),
LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),
SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or
ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42), and
a pharmaceutically acceptable carrier.

59. **(Unchanged)** The pharmaceutical composition of claim 58, wherein the peptide has less
than 20 amino acids.

60. **(Twice Amended)** A conjugate comprising

(a) a molecule, which comprises:

a polypeptide an having no more than a total of two single amino acid
substitutions, deletions or insertions at the corresponding positions as in a
CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),
LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),
QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO: 3),
KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or
LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35) or

has no more than a total of one single amino acid substitution, deletion or
insertion at the corresponding amino acid positions as in a CTL epitope
which is

DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),
LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),
SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or

E2 cont.

ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42);, and

(b) a substance selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a solid matrix, a carrier and an additional molecule of (a).

61. **(Unchanged)** The conjugate of claim 60, wherein said carrier comprises an immunogenic lipid or protein.

E3

62. **(Twice Amended)** A conjugate of claim 60 comprising two molecules, each comprising:
a polypeptide no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),

LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),

QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO: 3),

KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or

LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35) or

has no more than a total of one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),

LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),

SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or

ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42).

63. **(Unchanged)** The conjugate of claim 62, wherein at least one of said molecules comprises at least eight amino acids and less than 50 amino acids.

64. **(Unchanged)** The conjugate of claim 62, further comprising a T helper epitope.

65. **(Unchanged)** An isolated molecule comprising a polypeptide that induces an hepatitis C virus (HCV)-specific response in cytotoxic T lymphocytes having a sequence that has

(a) no more than a total of two single amino acid substitutions, deletions or insertions at the corresponding amino acid positions in a CTL epitope which is

LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2),

QLRRHIDLLV (E1₂₅₇₋₂₆₆; SEQ ID NO:3),

KLVALGINAV (NS3₁₄₀₆₋₁₄₁₅; SEQ ID NO:28), or

LLFNILGGWV (NS4₁₈₀₇₋₁₈₁₆; SEQ ID NO:35), or

(b) has no more than one single amino acid substitution, deletion or insertion at the corresponding amino acid positions as in a CTL epitope which is

ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1),

DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54),

LLCPAGHAV (NS3₁₁₆₉₋₁₁₇₇; SEQ ID NO:26),

SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), or

ILDSFDPLV (NS5₂₂₅₂₋₂₂₆₀; SEQ ID NO:42),

wherein said polypeptide comprises at least eight amino acids and less than 50 amino acids, wherein said selected CTL epitope maintains an

XaaLeuXaaXaaXaaXaaXaaXaaVal or

XaaLeuXaaXaaXaaXaaXaaXaaXaaVal motif,

with the proviso that (a) when said selected CTL epitope is SLMAFTAAV (NS4₁₇₈₉₋₁₇₉₇; SEQ ID NO:34), then said polypeptide comprises from at least eight amino acids to less than 25 amino acids, (b) when said selected CTL epitope is LLALLSCLTV (Core₁₇₈₋₁₈₇; SEQ ID NO:2) then said molecule comprises at most ten amino acids, and (c) when said selected CTL epitope is DLMGYIPLV (Core₁₃₂₋₁₄₀; SEQ ID NO:54), then said molecule comprises at most nine amino acids.

66. (Unchanged) The method of claim 52, wherein the peptide comprises the sequence which is ADLMGYIPLV (Core₁₃₁₋₁₄₀; SEQ ID NO:1).